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Morpho-Histological architecture of various cells with special emphasis on the seasonal variations of gonadotrophs in the pituitary gland of *Notopterus notopterus* (Pallas, 1769) in relation to testicular maturation

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Abstract
The pituitary gland of *Notopterus notopterus* (Pallas, 1769) was situated in a shallow pit at the floor of the skull and attached to the infundibulum. On the basis of tinctorial properties the cell types of pituitary were indentified which were distributed in the rostral pars distalis (RPD) proximal pars distalis (PPD) and pars intermedia (PI) in different proportions. The three zones were innervated by the narrow process of neurohypophysis. The acidophilic prolactin cells (PRL) and adrenocorticotropic cells (ACTH) were observed in the RPD which were stained with acid fuchsin and orange G by respectively. The basophilic gonadotrophs (GTH) and thyrotrophs (TSH) were distributed in the middle PPD and reacted positively to aniline blue, periodic acid Schiff's (PAS) and aldehyde fuchsin (AF) stain. The only acidophilic somatotrophs (STH) cells were also distributed in between GTH in PPD region which were positively stained with PAS and acid fuchsin. The GTH and TSH cells exhibited both quantitative and qualitative variations during the testicular cycle. The seasonal changes in the testes of *N. notopterus* have been described according to the variations in GSI values and frequency percentages of the different germ cells occurring in the testicular lobules. The sequential changes of GTH and TSH cells and testicular activities were correlated well during different reproductive phases.

Keywords: Architecture various cells, Seasonal variation, Gonadotrophs, Pituitary, *Notopterus notopterus*

1. Introduction
The fishes reproduce in their natural environment to produce offspring and continue their progeny. Both environmental and hormonal factors are extremely important in regulating reproductive behaviour and spawning in fishes. Various central mechanisms translate environmental cues into chemical messengers which function to activate and maintain the reproductive organs. In this regard the functional relationship between the hypothalamus and pituitary gland is important. The pituitary has a central role in controlling gonadal activity and the identification and distribution of the cell types in the pituitary gland of the different teleosts have attracted some investigators from the histochemical, ultrastructural and immuno-cytochemical techniques [1, 2, 3]. They emphasized that the secretory cells of the pituitary gland show different patterns of distribution in RPD, PPD and PI. Most of the authors have pointed out that single type of gonadotropin secreting cell are present in some fish species [3, 4]. However, two GTH cell types have also been described in other teleost species by Mousa and Mousa [5] in *Mugil cephalus.* The spermatogenesis is a very active process and the testicular cycle in a majority of freshwater teleosts which are seasonal breeders undergo remarkable changes during various periods of the season [6, 7, 8]. As far the knowledge is concerned, there are few earlier studies regarding the seasonal changes of GTH cells in the adenohypophysis of the freshwater teleosts [9, 10]. Hence the aim of the present study is to identify and localize the different cell types with special emphasis to GTH cells in the pituitary gland of male *Notopterus notopterus* (Pallas, 1769) at all stages of testicular development. This feather back is very much popular as food fish due to its high nutritive and palatable qualities.

2. Materials and Methods

Ten adult male of *N. notopterus* (average length 22.5 ± 1.20 cm and mean body weight 75 ± 2.25 g) were procured fortnightly throughout the year during December, 2013 to November, 2014 from a particular stocking pond located at Burdwan, West Bengal, India in order to avoid ecological variations in different water bodies that can affect the functional status of pituitary and testes.

2.1. Gonadosomatic Index (GSI)

Ten male fishes were collected every month during December, 2013 to November, 2014 and the total body weight of the fishes and the total weight of the testes were taken every month to calculate the GSI from the following formula:

$$GSI = \frac{\text{Weight of the gonads}}{\text{Weight of the fish}} \times 100$$

2.2. Histological and histochemical methods

After decapitation of the fish the pituitary glands along with the entire brain were fixed in Bouins fluid and Eltman's fixatives for 18h. Pieces of testicular tissues were also fixed in Bouins fixative for the same period of time. The tissues were then placed in 70% ethanol and subsequently dehydrated with increasing ethanol concentration followed by acetone and cleared in benzene. Tissues were then embedded in paraffin wax (56-58°C melting point). The testes and mid-sagittal sections of the pituitary glands were cut at 4µm thickness using a Leica RM 2125 RT microtome. Deparaffinized sections were stained by adopting various techniques which are as follows.

2.2.1. Periodic Acid Schiff's (PAS) technique of McManus [11] using orange G as the counter stain (PAS-OG) for demonstration of gonadotrophs, thyrotrophs and melanotrophs.

2.2.2. Mallory's triple stain (MT) (Mallory) [12] for demonstration of gonadotrophs prolactin, corticotrophs and thyrotrophs.

2.2.3. Chrome alum haematoxylin phloxine (CAHP) after Gomori [13] for demonstration of corticotrophs, somatotrophs and prolactin cells.

2.2.4. Alcian Blue-Orange G-Acid fuchsin (AB-OFG) after sliders [14] for demonstration of somatotrophs, gonadotrophs and thyrotrophs.

2.2.5. Alcian Blue (AB) (pH 0.2 and 2.5) combined with PAS-OG by acid permanganate oxidation as adopted by Herlant [15] for demonstration of thyrotrophs, gonadotrophs, prolactin and somatotrophs.

Sections of testes were also stained with Iron alum haematoxylin (IAH), Delafield's haematoxylin and eosin (HE) and Mallory's triple stain (MT) for identification of different germ cells including interstitial cells. After staining the sections were dehydrated through ascending series of ethanol, cleared in xylene, mounted permanently with DPX and then examined under binocular microscope. From the histological preparation, the diameter of different cells particularly GTH cells were calculated with the help of reticulo-micrometer and ocular micrometer. The percentage of occurrence of the GTH cells during different reproductive phases were measured calculating the number of GTH cells per hundred cells of five different sites for a particular fish on a total of ten different fishes and then averaging their number for a particular phase. The diameters of the various spermatogenetic cells of the fish were measured in a total of twenty cells per fish.

3. Results

The pituitary gland of *N. notopterus* was attached ventrally to the infundibulum of the brain. In the mid-sagittal sections and based on the histological features of its cell types, the adenohypophysis was divisible into three component parts: the frontal rostral pars distalis (RPD), the middle proximal pars distalis (PPD) and the distal pars intermedia (PI). Although there was no sharp demarcation between these zones but a narrow line of penetration of the axonal fibres of neurohypophysis ramified between RPD, PPD and PI. Various cell types had been identified in the RPD, PPD and PI on the basis of their staining intensities with different staining methods.

3.1 Distribution of different types of cells in the pituitary gland

3.1.1. Rostral Pars Distalis (RPD)

Two distinct cell types were mainly identified in this region. The rounded acidophilic prolactin cells (PRL) occupied the major part of the RPD exhibited positive reaction in the cytoplasm with acid fuchsin and orange G but negative to PAS and aniline blue stain. During the maturation phase some aniline blue positive basophilic cells were found to be dispersed among the PRL cells were identified as the gonadotrophs (GTH) cells (Fig. 1) In *N. notopterus* oval or elongated corticotropic cells (ACTH) were scattered along with other cell types of the RPD. These cells had strong affinity to acid fuchsin and erythrosin (Fig. 5) but negative to AF and aniline blue.

3.1.2. Proximal pars distalis (PPD)

In *N. notopterus* the main cell types of PPD were of basophilic in nature and contained cytoplasmic granules stained purple colour with PAS-OG and could be recognized as gonadotrophs (GTH) or cyanophil-I cells and thyrotrophic cells (TSH) or cyanophil-II cells (Figs. 2 and 3) During maturation phase cytoplasm of GTH and TSH cells showed purple blue colour with AB-PAS-OG and exhibited navy blue colour with aniline blue of Mallory's triple stain. The GTH and TSH in *N. notopterus* showed significant hypertrophy during spawning season (Figs. 10 and 11). The scattered rounded or oval acidophil cells in the PPD zone were identified as the somatotrophs (STH). The cytoplasmic granules of these cells stained orange red with Mallory's triple stain and AB-PAS-OG but negative to PAS, AB and aniline blue (Figs. 5 and 7).

3.1.3. Pars intermedia

The cells exhibited amphiphilic reactions found in this region were identified as the melanotrophs (MSH) cells. These cells were PAS-OG, positive, generally round in shape and had rounded nucleus (Fig. 15).

3.1.4. Chromophobe cells

They were small in size, spherical or oval in shape with small ecentric nuclei and considered as degranulated state of various chromophil cells. These cells were located sparsely in RPD and PPD region (Fig. 14).

3.2 Spermatogenesis

The testis of *N. notopterus* was angular shaped, single lobe, whitish coloured situated at the left, laterally position. The

seminiferous tubules were generally varied shapes and sizes. The event of spermatogenesis in *N. notopterus* had been divided into five distinct stages, viz., spermatogonia (stage 1), primary spermatocytes (stage 2), secondary spermatocytes (stage 3), spermatids (stage 4) and spermatozoa (stage 5). The characteristic features of the aforesaid stages were as follows:

3.2.1. Spermatogonia
These were largest ones of all the spermatogenetic cells occurred singly or in the nests attached to the lobule boundary wall. These cells were more or less spherical in shape with a rounded nucleus stained with haematoxylin and chromophobic cytoplasm. The diameter of these cells varied from 6.4 μm × 5.0 μm to 9.0 μm × 7.5 μm (Fig. 4) the nucleus (diameter 1.6 μm to 2.84 μm) was much darker and nucleoli were central to the nuclei (Fig. 4).

3.2.2. Primary spermatocyte
The primary spermatocytes were almost oval or round in shape and contained relatively lesser amount of chromophobic cytoplasm and the nucleus was deeply stained with haematoxylin. The diameters of these cells varied from 8.32 μm × 6.4 μm and the diameter of the nucleus measured 4.32 μm × 4.76 μm (Fig. 4).

3.2.3. Secondary spermatocyte
The secondary spermatocytes were smaller than primary spermatocytes. The nuclei were highly condensed and the cytoplasm of the cells was difficult to distinguish. The diameter of the nucleus was approximately 3.2 μm × 4.8 μm. They lasted for a short duration and thereafter produce spermatids (Fig. 9).

3.2.4. Spermatids
These cells remained in dense aggregation within the lumen of the lobules. The nucleus was almost crescent shaped and densely stained with haematoxylin. The diameters range 3.2 μm to 4.16 μm. They were undergone series of transformation resulted in the formations of mature spermatozoa (Figs. 4 and 8).

3.2.5. Spermatozoa
The spermatozoa were occurred in the central position of lumen in the lobules. The diameter of the sperm nucleus was 2.08 μm and had strong affinity to haematoxylin (Figs. 8, 9 and 12).

Interstitial cells
The cells were round, oval in shape and lied in the interlobular spaces (Figs. 8, 9 and 12). The interstitial cells were undergone variations in shape and size during different reproduction phases.

3.3. Seasonal changes in the gonadotropic cells in relation to testicular maturation
In the present observation the seasonal changes in the gonadotropic cells in the pituitary and different germ line cells in the testes were described on the basis of shape, size and frequency percentage of GTH cells, GSI and various germ cells in the testicular lobules. Accordingly, the reproductive cycle in *N. notopterus* might be grouped into growth, maturation, spawning and post-spawning phases which were as under:

Table 1: *Seasonal variations in the GSI of male Notopterus notopterus*

Maturity stages	Months	Mean GSI ± SE
Growth phase	December	0.32 ± 0.05
	January	0.56 ± 0.22
	February	0.80 ± 0.13
Maturation phase	March	0.861 ± 0.19
	April	0.92 ± 0.8
	May	1.02 ± 0.19
Spawning phase	June	1.16 ± 0.11
	July	1.21 ± 0.30
	August	1.11 ± 0.12
Post-spawning phase	September	0.91 ± 0.20
	October	0.79 ± 0.03
	November	0.14 ± 0.04

Table 2: The cellular diameter of GTH cells and the percentage of occurrence in the PPD of male pituitary of *N. notopterus* during different reproductive phases (December, 2013 to November, 2014).

Maturity stages	Months	GTH cell diameter (μm)	GTH % in PPD
Growth phase	December	6.40 ± 0.18	22.9
	January	6.58 ± 0.32	26.8
	February	6.64 ± 0.51	29.6
Maturation phase	March	8.02 ± 0.55	38.2
	April	8.50 ± 0.30	40.4
	May	8.46 ± 0.21	41.9
Spawning phase	June	9.20 ± 0.42	45.0
	July	8.82 ± 0.65	43.8
	August	8.78 ± 0.22	38.5
Post-spawning phase	September	8.62 ± 0.38	26.5
	October	7.20 ± 0.26	24.2
	November	6.32 ± 0.44	22.6

3.3.1. Growth phase (December to January)
The cytomorphological changes in the GTH and TSH cells of the pituitary exhibited a close relationship to the changes in the testicular follicles during their growth. The GTH cells were mainly distributed in the ventral portion of PPD. The rounded nuclei of GTH cells lied more or less middle of the cell. These cells were reacted with aniline blue by Mallory's triple stain (Fig. 3). Considerable number of, STH and TSH cells were discernible in the PPD zone. During this phase the average diameter of the GTH cells were calculated to be 6.40 ± 0.18 in December to 6.64 ± 0.51 in February. An increase in the number and activities of GTH cells during the end of growth phase occurred at the time of differentiation of testicular germ cells (Fig. 3). In the present investigation in testes the value of GSI ranged from 0.32 ± 0.05 to 0.80 ± 0.13 (Table 1). Spermatogonial cells were the principal cell types encountered during December. Primary, secondary spermatocytes and spermatids were noticed during January and February (Fig. 4).

3.3.2. Maturation phase (March to May)
During this phase the GTH cells attained their maximum size and occupied almost the entire PPD region and to some extent also in the bonder of RPD region (Fig. 5) the increment in the cytoplasmic content and volume of GTH cells surrounding blood vessels were clearly detected during this phase (Fig. 6). The GTH cells reached its maximum cytoplasmic volume with homogenous mass and the mean cellular diameter of the cells is 8.02 ± 0.55 in March to 8.46 ± 0.21 during May.

On the other hand, the increment of cytoplamsic volume was also noticed in the TSH and STH cells (Fig. 7) encircled orange G positive STH cells in the PPD region. During this

phase when the testes entered into the maturation phase, the GSI aligns between 0.86 ± 0.19 to 1.02 ± 0.19 (Table 1). The diameter of the tubules increased considerably. All types of spermatogenic cells appeared within the tubules (Fig. 9). Spermatogonia decreased in number but the numbers of spermatids and spermatozoa increased (Fig. 8). There was an apparent reduction of the interlobular connective tissue probably due to the increased in size of the lobule (Figs. 8, 9). During the end of this phase the seminiferous lobules were packed with cysts of spermatids and spermatozoa leaving few spermatogonia and secondary spermatocytes (Figs. 8 and9). The interstitial cells became active and the size of the cells varied from 4 to 6 µ (Figs. 8 and 9).

3.3.3. Spawning phase (June to August)

In the early spawning phase slight increased in the average diameter of GTH cells was noticed. It had a value of 9.20 µm ± 0.42 in June (Fig. 10). However, decreased trend was noticed in July and August when the diameter of GTH cells was recorded to be 8.82 µm ± 0.65 in July and 8.78 µm ± 0.22 in August (Table II). The cytoplasm of GTH cells and TSH cells was greatly reduced and the large nucleus occupied the greater part of the cytoplasm (Fig. 11).

In the testes the GSI value was recorded to be 1.16 ± 0.11 and 1.21 ± 0.30 during June and July respectively. However, declined trend of GSI was observed from August when the GSI is noticed to be 1.11 ± 0.12 (Table I). The diameter of testicular lobules increased and the boundary wall was extremely thin (Fig. 12). During this phase the lobules are uniformly packed with spermatozoa although few cysts contained spermatids were also observed (Figs. 12, 13). The interstitial cells became hypertrophied and the sizes of the cells varied from 5.0 µm × 7.5 µm to 8.0 µm × 10.5 µm (Figs. 12, 13).

3.3.4. Post-Spawning phase (September to November)

The size of the GTH cells decreased considerably from September onwards and the average size of the GTH cells were calculated to be 8.62 µm ± 0.38 in September which further decreased to 7.20 µm ± 0.26 in October. Degranulation of the GTH cells was continued. In the PPD region the STH cells were considerably increased in number and some chromophobe cells were also discernible in between GTH and TSH cells (Fig. 14). In the PI region the amphiphilic rounded MSH cells were more numerous towards the blood vessels (Fig. 15) during this phase.

In the testes the GSI value declined to 0.91 ± 0.20 to 0.14 ± 0.04 during this period. The diameter of the tubules decreased and the lobule boundary wall gradually became thicker. Residual spermatozoa were dispersely arranged in the lumen, cysts of spermatids still present. The lobule boundary wall is lined with spermatogonial cells. The interstitial cells are still prominent in between lobules adjacent to blood vessel (Fig. 16).

Figs. 1-16: Photomicrographs of sections of pituitary and testes during growth, maturation, spawning and post-spawning phases of *N. notopterus.*

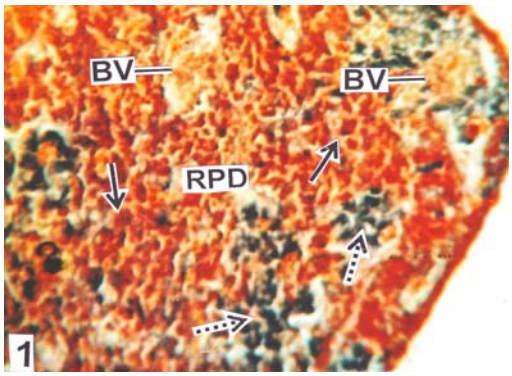

Fig 1: RPD region of pituitary gland during growth phase showing the maximum distribution of PRL cells (solid arrows), GTH cells (broken arrows) dispersed among PRL cells adjacent to blood vessels (BV) (CAHP) × 150X.

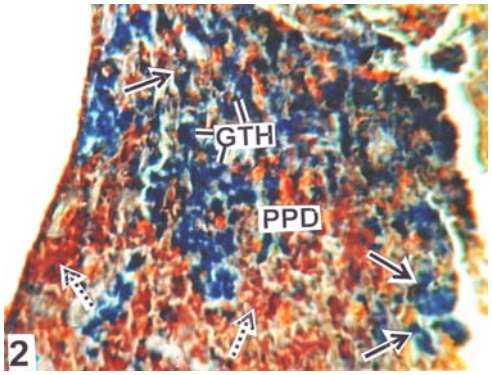

Fig 2: PPD region of pituitary during end by growth phase showing aniline blue positive densely populated GTH cells in the middle region. Note TSH cells (solid arrows) and STH cells (broken arrows) in between GTH cells (MT) × 150X.

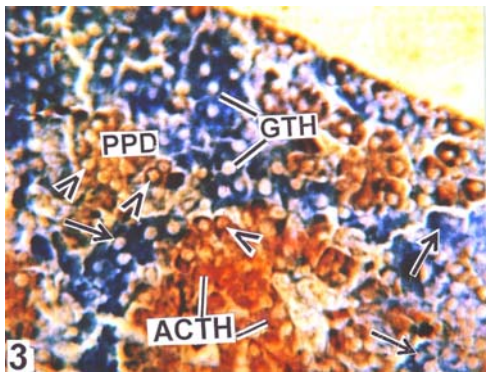

Fig 3: PPD region of pituitary gland showing intense cytoplasmic reaction of aniline blue in the GTH cells and TSH cells (arrows) during end of growth phase. Note the presence of ACTH and STH cells (arrow heads) in between (MT) × 400X.

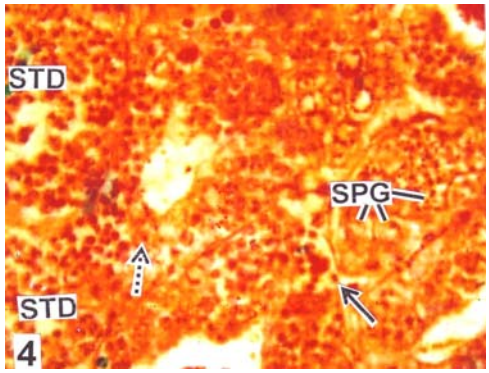

Fig 4: Testicular lobules with spermatogonial cells (SPG) and broken arrow and increment of spermatids (STD) during late growth phase. Solid arrow indicates interstitial cells (IC) (HE) × 400 X.

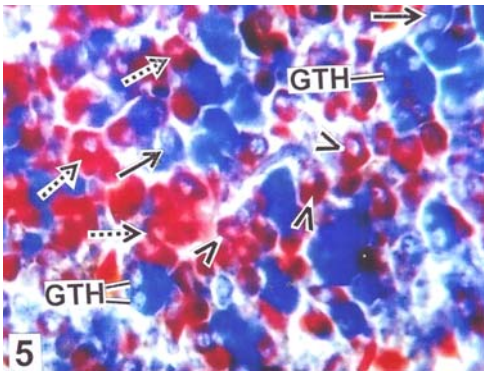

Fig 5: Border of RPD and PPD region during maturation phase showing acid fuchsin positive PRL cells (arrow heads) and ACTH cells (broken arrows) Note aniline blue positive GTH and TSH cells (solid arrows) (MT) × 600X.

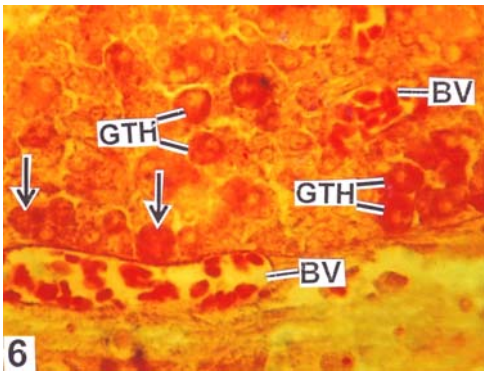

Fig 6: Showing fully matured PAS positive GTH cells and TSH cells (arrows) adjacent to blood vessels (BV) during maturation phase (PAS-OG) × 600X.

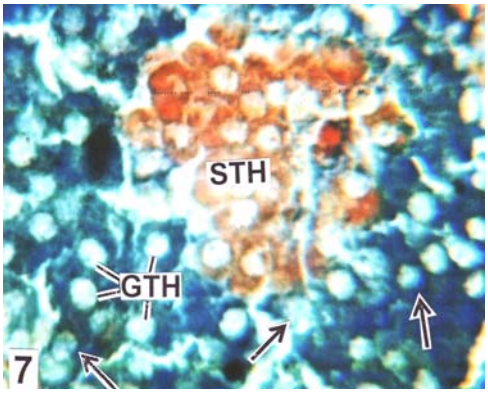

Fig 7: Showing quantitative increment of alcian blue positive GTH and TSH cells (arrows) encircling orange G positive STH cells in the PPD region during maturation phase (AB-OFG) × 600X.

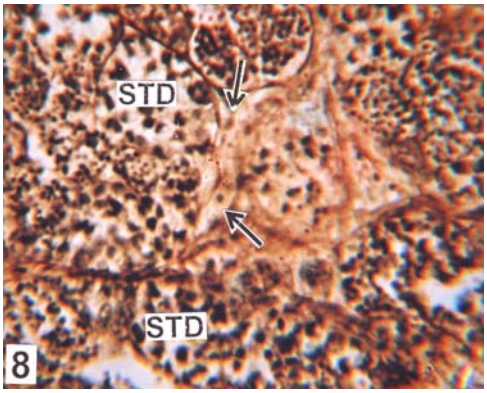

Fig 8: Testicular follicles packed with spermatozoa and cysts of spermatids (STD) during maturation phase. Note the presence of interstitial cells (arrows) in the inter follicular space (IAH) × 400X.

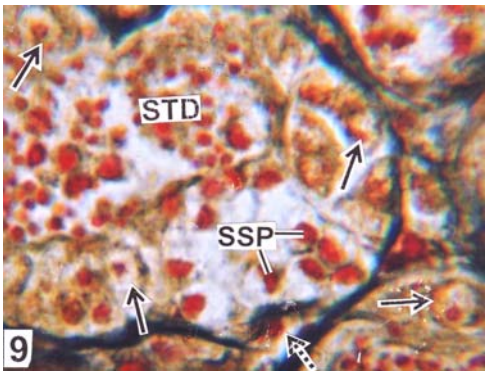

Fig 9: Higher magnification of testicular follicles during maturation phase showing cyst of spermatids (STD), secondary spermatocyte (SSP) and few spermatogonia (solid arrows). Broken arrow indicates interstitial cell (MT) × 1000X.

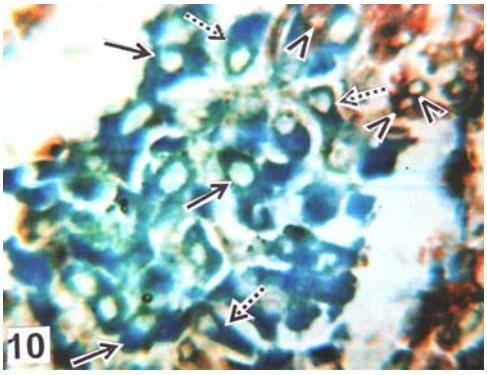

Fig 10: PPD region of pituitary showing hypertrophy of GTH (solid arrows) and TSH cells (broken arrows) with densely stained cytoplasm during spawning phase. Note some PAS positive STH cells (arrow heads) (AB-OFG) × 600X.

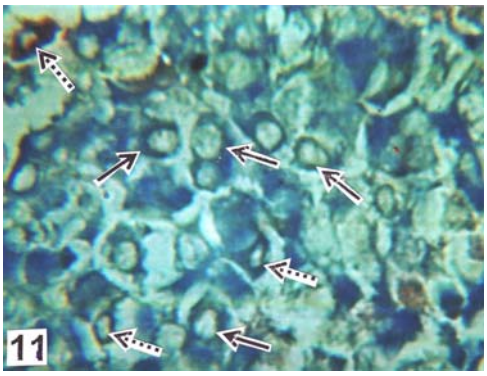

Fig 11: PPD region of pituitary showing reduced cytoplasmic content in the hypertrophied GTH (solid arrows) and TSH (broken arrows) cells during end of spawning phase (AB-OFG) × 600X.

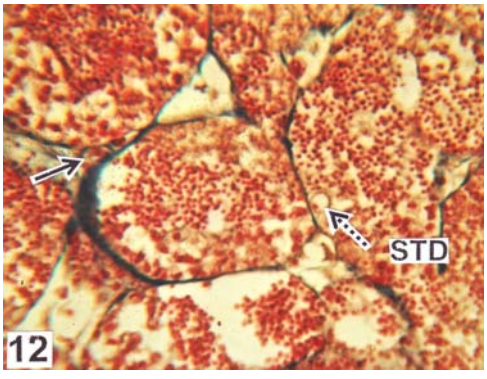

Fig 12: Thin walled testicular follicles packed with spermatozoa within the lumen during spawning phase. Note cysts of spermatids (STD) along the border of follicles. Note interstitial cells (solid arrow) and spermatogonial cell (broken arrow) (HE) × 400X.

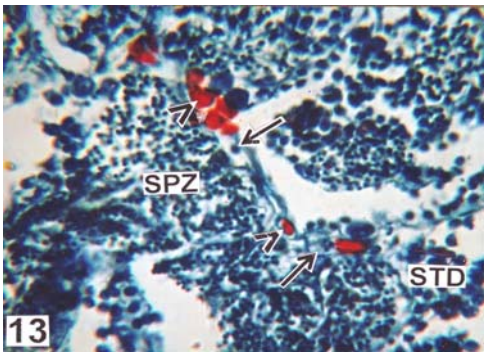

Fig 13: Dense population of spermatozoa (SPZ) and cysts of spermatids (STH) during end of spawning phase. Note the presence of interstitial cells (arrows) adjacent to blood vessels (arrow heads) (MT) × 600X.

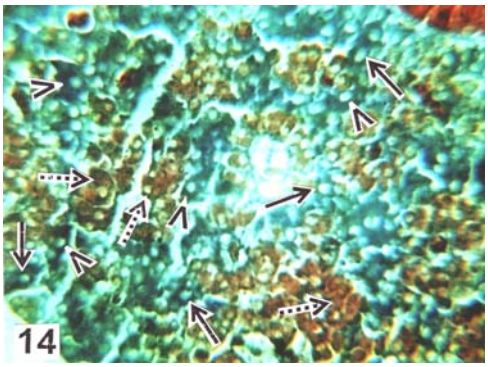

Fig 14: Reduction of GTH (solid arrows) and TSH (arrow heads) cells in the PPD region of pituitary during post-spawning phase. Note the presence of STH cells (broken arrows) in between GTH cells (AB-OFG) × 400X.

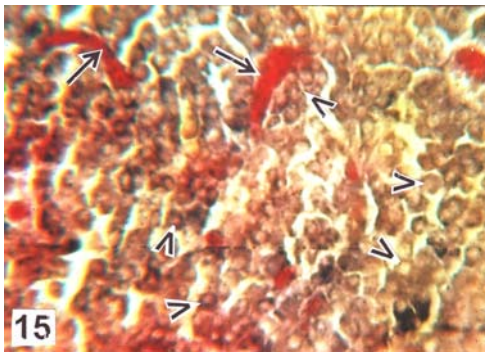

Fig 15: PI region of pituitary showing reddish orange MSH cells (arrow heads) adjacent to blood vessels (arrows) during post-spawning phase (PAS-OG) × 600X.

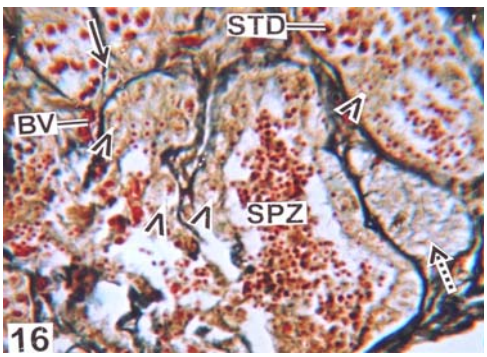

Fig 16: Testicular lobule showing thick boundary wall during post-spawning phase having residual spermatozoa (SPZ) in the lumen and cysts of spermatid (STD). Note spermatogonial cells (arrows heads) along the border of lobules. Note also cluster of spermatogonial cells (broken arrow). Solid arrow indicates interstitial cell adjacent to blood vessel (BV) (MT) × 600X.

4. Discussion

In teleost the hypophysis is known to vary greatly in topography, size and mode of attachment in nervous ramification as well as histoarchitecture [16]. In the present observation in *N. notopterus* different types of hormone producing cells are arranged in a mosaic pattern within the rostral pars distalis (RPD), proximal pars distalis (PPD) on the basis of staining reaction in the cytoplasmic content. The nerve fibres of neurohypophysis is extremely regular, so that to a large extent differentiation of cell types in tinctorial techniques becomes easy. Joy and Sathyanesan [17, 18], Jafri and Ensor [19] identified precisely the different cell types situated in the pituitary of a few teleosts. They categorised various cell types in the teleostean pituitaries on the basis of the staining reaction in the cytoplasmic content adopting different staining technology. In the present investigation the prolactin (PRL) cells are provided with acid fuchsin and azocarmine staining granules formed the major component in the RPD. This finding is in agreement with those of Sage and Bern [20], Joy and Sathyanesan [17] in different teleosts. The prolactin like hormone may be considered necessary for osmoregulation in fish under study. Ball and Baker [21] believed that prolactin hormone acts as a carrier for sodium ion transport in the chloride cells of the gills and is essential for osmoregulation. The second types of spherical or oval acidophils comparable to corticotropic (ACTH) in the RPD which are densely stained with acid fuchsin erythrosin are interlocated between the PRL cells in *N. notopterus.* Zaki *et al.* [22] Assem and El-Boray [23] reported that the ACTH cells are generally found at the interphase between PRL cells and the neurohypophysis and occurred in groups. According to Mandal and Sinha [24] the ACTH cells are lead haematoxylin positive and are located in the RPD bordering the neurohypophysis and occurred in groups in *Catla catla*. In the present study, in *N. notopterus* the cyanophil-I or GTH cells formed the main bulk of cells of the PPD. These cells are comparatively large angular in shape and displayed purple colour in PAS-OG stain and purple blue colour in AB-PAS-OG stain but negative to CAHP and acid fuchsin stain and these cells located mainly in the middle part of PPD. Few GTH cells are also located along the border of the pars intermedia (PI). In *Oncorhynchus kisutch* the GTH cells are located in the PPD arranged as cords of cells [25]. Many authors confirmed cyanophil cells of various types in the different regions of teleostean pituitaries. Jose and Sathyanesan [26] recorded two types of cyanophil cells in the border of RPD and PPD as well as in the PPD proper of *Labeo rohita*. In the present observation cyanophil-II cells or TSH cells in *N. notopterus* stained navy blue colour with aniline blue and are located in the PPD intermingled with cyanophil-I cells. Ali [1] showed that the thyrotrophs exhibit navy blue colour while the gonadotrophs impart red colour when AB-PAS-OG-ACF stain is employed in the pituitary of roach. Both the TSH and GTH cells show a significant hypertrophy during the breeding season as also advocated by Joy and Sathyanesan [18]. In *N. notopterus* the only acidophils of the PPD region considered as STH cells stained positively with PAS-OG and acid fuchsin and negatively with AF. These STH cells are arranged in groups between the GTH and TSH cells. Similar pattern of distribution of STH cells are also indicated by Narayan *et al.* [27] in *Valamugil cunnesius*; in *O. Kisutch* by Leatherland and Sonnstegard [25]. In the present investigation the MSH cells are amphiphilic in nature and located sparsely in the PI region in *N. notopterus*. These cells are stained with PAS-OG and CAHP. PAS positive MSH cells have also been described in pars intermedia of *Liza parsia* [28].

In many teleosts, a correlation between the gonadotropic cells

and the gonadal cycle have been observed showing the hyperplasia, hypertrophy and other signs of increased activity of these cells in association with the maturation of the gonads [9, 21]. The GTH cells of the in PPD *N. notopterus* exhibit their prominence having dense basophilic cytoplasm stained with PAS, AB and aniline blue during the maturation of testes. During the growth phase the low active condition of the GTH cells is well coincidence with the increase in spermatogonial cells and with absence of spermatozoa. At the end of maturation phase and prior to spawning the cyanophil-I or GTH cells increase in their number. During this period, the GTH cells form the major component of the PPD. Degranulation and vacuolization is clearly observed in the GTH cells appeared to be concomitant with spermiation. This clearly indicates that the GTH is an essential prerequisite for the spermiation. The proliferation of different testicular cells as revealed by GSI showed an increase in the maturation stage and attains peak values in the late maturation or early spawning phases. During the spawning phase, most of the GTH cells became vacuolated although some might be continued to load with cytoplasmic materials. At this time majority of semniferous tubules are packed with spermatozoa and cysts of spermatids although few spermatogonia might persist. This is in conformity with the findings of Krishnan and Diwan [29] and Gaber [30]. Joy and Sathyanesan [17] have clearly demonstrated that in *Clarias batrachus*, the basophils in the pituitary exhibited gradual increase in their number when the gonads start maturing and in those with ripe gonads the basophils form the major component of the PPD.

During the post-spawning phase the GTH cells are more or less inactive and decreased its average number and size accompanied by an increase in the number of spermatogonial cells. In *N. notopterus* another basophilic cells i.e. TSH cells or cyanophil-II cells followed a more or less similar cycle as the gonadotrophs, suggested a possible synergistic association of the two in gonadal maturation. During maturation and spawning phases these cells appear granulated having dense cytoplasmic stain.

5. Conclusion

In *N. notopterus* prolactin cells (PRL), corticotropic cells (ACTH) and few basophilic gonadotropic cells (GTH) were arranged in a mosaic pattern within the rostral pars distalis (RPD). However, in proximal pars distalis (PPD) region was densely occupied by GTH and thyrotropic cells (TSH) which showed significant hypertrophy during maturation and spawning phases. No significant changes of the activity of ACTH, PRL, somatotropic (STH) cells and melanocyte hormone secreting cells (MSH) were observed during different reproductive phases.

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7. References

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